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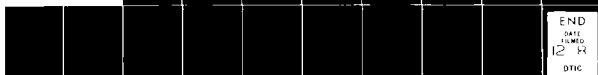
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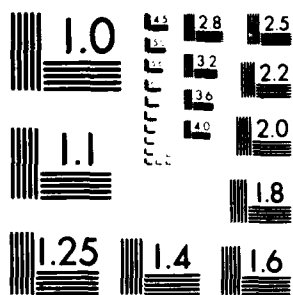
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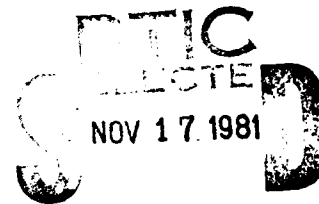
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BIOPHYSICS OF WATER: A WORKING CONFERENCE

P. T. BEALL\*

15 SEPTEMBER 1981

\* Baylor College of Medicine, Houston, TX

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BIOPHYSICS OF WATER: A WORKING CONFERENCE

INTRODUCTION

A working conference on the Biophysics of Water was held at Girton College, University of Cambridge, England, on June 29 - July 3, 1981. Support was provided by the Office of Naval Research and by the German Biochemical Society. Joint organizers were Dr. Felix Frands, Department of Botany, University of Cambridge, and Dr. John Finney, Department of Crystallography, Birkbeck College. The general aim of the conference was to bring together an international group of physicists, chemists, biochemists, physiologists, and microbiologists to discuss the relationships between the properties of water and cell function/physiology.

An international organizing group chose four major areas of research to serve as topics. The subjects were: Molecular Hydration and Biological Function, Surface Forces in Biological Systems, Water as an Agent of Homeostasis in Organisms, and Physiological Water Stress. Special evening lectures on the current state of the art on water and forces between surfaces in electrolyte solutions were also given. The final day featured the presentation of alternative views on the role of water in cell function. A summary discussion of the meeting concluded the conference.

The format of the conference was designed to provide the greatest possible opportunity for open discussions and scientific interactions among the participants. A Gordon Conference-style meeting was arranged at Girton College. Each day's session began with a general plenary lecture summarizing the present state of knowledge and problems in an area. Short introductions to a limited number of selected posters on the topic were followed by an hour devoted to observation of the posters that had been on view since the previous afternoon. The meeting then reconvened for an hour's discussion of the posters. Afternoons were open for individual interaction. At four o'clock tea was served, followed by a discussion led by a panel of experts on the day's topic. Each panel member had 20 minutes to deliver a prepared statement of his views and particular approach to the problem and to suggest future lines of inquiry. An active discussion of the panel's comments and the topic of the day followed, during which the audience members had the opportunity to ask questions, deliver comments, and show up to three slides to support their arguments. Each evening was given to a presentation by a feature lecturer. Discussions for the day were recorded and summarized for publication by a reporter.

The conference was attended by approximately 110 participants from 17 countries. One reason the conference was held in England was to provide an opportunity for many of the Eastern European scientists engaged in water research to attend. Although at the last moment Russian scientists and some scientists from Rumania were not able to attend, there were still 9 scientists from Yugoslavia and Rumania present as well as 5 scientists from Australia and New Zealand.

Dr. Max Perutz, Nobel prize winner for the X-ray diffraction study of hemoglobin, was a special guest lecturer. Perutz discussed his early research (in the 1930s) on X-ray diffraction studies of ice formation in glaciers, and the development of a theory of why skis have to be waxed. In reference to glaciers, he pointed out that while ice is not elastic, under critical pressures it becomes plastic. Glaciers are tall enough to create sufficient pressures near their base to cause ice to flow. Ice crystals are large in glaciers because thaw-melt cycles favor the survival of large crystals that grow bigger and bigger with the melt water of other crystals as they refreeze. Regarding skis, he noted that wax on old-fashioned wooden skis prevents the conduction of heat away from the bottom of the ski. The friction of motion melts a thin film of water upon which the ski actually travels. These findings explain why modern skis are not made of metal, but rather of non-conducting plastics.

#### MOLECULAR HYDRATION AND BIOLOGICAL FUNCTION

The plenary lecture on the first day was delivered by Dr. Roger H. Pain of the University of Newcastle, England. He stressed the ubiquitous nature of water in all biological systems. As a major biological solvent, water is responsible for the transport of materials into and out of cells, the hydrated properties of ions and metabolites, and the shapes of hydrophilic molecules in solution. Water may also act as a poor solvent for hydrophobic regions of macromolecules. Mutual hydrophobia of structural groups may be responsible for the 3-dimensional structure of macromolecules, where the strength of the hydrophobic interaction is proportional to the area of the polar molecules. Pain stressed that cells are not dilute solutions and do not observe the chemistry of dilute solutions. They are highly organized and integrated systems in which water may play a structural and stabilizing role. Some large molecular aggregates such as viruses and microtubules, for example, utilize water hydrogen bonding in stabilizing their polymerized form. The denaturation of proteins such as penicillinase can be explained by the effects of urea or guanidine on the properties of the water rather than the protein. A sometimes forgotten role of water in biological systems is that of a lubricant. In glycoprotein mucus layers, it coats the lungs and digestive tract. Loss of water or imbalance in the gel-sol properties of mucus can have serious consequences. Water's properties as a good solvent lead to the movement of metabolites; as a poor solvent it contributes to structure; and in combination with macromolecules it becomes a lubricant. All these effects depend on water-macromolecular hydration.

Two important points that were raised by Pain came up repeatedly throughout the conference. The first was that, while certain denaturants such as urea or guanidine can be used to cause conformational changes in proteins by their own interactions with water, it is not clear why mixtures of urea and guanidine in approximately equal molar concentrations tend to cancel each other's effect leaving the protein unchanged. Secondly, it was pointed out that while we can measure the so-called "hydrophobic

effect," we do not understand it. The  $\Delta H$  for insertion of a methane molecule into methane liquid is much less than the  $\Delta H$  for the insertion of a methane into water. The repulsion of the water matrix is quite high and is also very temperature dependent. In order to adapt and create a minimal  $\Delta H$  for biological chemistry, each species has selected some amino acid sequences that create the least disturbance of  $\Delta H$  in its own temperature range.

The following posters were presented in this section by the authors indicated: (1) Dynamical and Structural Aspects of Protein Hydration studied by High Resolution Neutron Scattering (Sir John Randall and H.D. Middendorf, Univ. of London, UK); (2) Comparative Studies of Different Models of the Solvent Effect on the Conformation of Biological Molecules (B. Robson, Univ. of Manchester, UK); (3) Correlation between Aqueous Solubility and Partitioning in Sephadex G-15 (A. Haglund and N.U.B. Marsden, Biomedicom, Uppsala, Sweden); (4) Characterization of the State of Water within Synthetic Membrane by Heat Capacity and Water Sorption Isotherm Measurements (W. Lukas and W. Pusch, Max-Planck Inst., Frankfurt); (5) Computer Simulation of Solvent Around Biomolecules (H. Savage, J.M. Goodfellow, J. L. Finney, P. Barnes, Birkbeck College, London, UK); (6) An Investigation of the Hydration of Lysozyme using a Direct Difference Infra-Red Technique (P.L. Poole and J. L. Finney, Birkbeck College, London, UK); (7) Water Bridges in Myoglobin (J.R. Grigera and I.G. Molinger, Argentina); (8) Low Temperature Haemoglobin Hydration in Dependence of Protein Concentration (J. Brnhas-Kraljevic and S. Maricic, Yugoslavia); (9) An NMR Study of Isotope Distribution and the State of Water in the Hydration Layer of DNA (M. DeVre, R.G. DeClerck, P. Lejeune, Inst. d'Hygiene, Brussels); (10) The Role of Solvation in Protein Structure Stabilization and Unfolding (S.N. Timasheff, Brandeis Univ., Waltham, MA)

These posters focused on such diverse techniques as neutron scattering; Monte Carlo modeling; solute partitioning; thermodynamics; infrared spectroscopy; NMR; and differential refractometry on the properties of water molecules, as forces in the formation of their tertiary structure as monolayers on their surfaces, and as the solvent for purified macromolecules. It has long been assumed that simplified model systems will yield more definitive answers on biological water than attempts to interpret data from the heterogenous complex cell. Of interest here is the fact that most of the model systems discussed at this conference were very large macromolecules such as DNA, hemoglobin, myoglobin, and lysozyme; molecules that actually exist in cells. Only some synthetic membranes and one cross-linked dextran were employed in attempting to draw conclusions applicable to biology. It is this writer's opinion that most researchers have come to the conclusion that effects determined for very simple models like polypeptides or nucleotides do not seem to give, in a predictable manner, the magnitude of effects seen for the macromolecules in solution or in the whole cell. Such a growing awareness among researchers in this field may lead to better techniques to examine complex model systems.

In the panel discussion, Timasheff was asked to comment on the preferential hydration of tubulin by water in solutions also containing

glycerol. He was asked if such preferential concentration of water around proteins could explain the cancellation effects of urea and guanidine as denaturing agents. It was speculated that urea and guanidine may act by two different mechanisms, and that when they are present in equal amounts, the two effects may cancel. The question of how to define "hydration water" or "bound water" also was raised in the first discussion session. The proposal was introduced that only those water molecules with an energy of association  $>KT$  should be called "bound," and that those  $<KT$  but not like bulk water should be called "intermediate" or "boundary" water. The possibility of using residence time to define bound water also was considered. The old idea of defining those nonfrozen water molecules visible to NMR (Nuclear Magnetic Resonance), as the "bound" water also was discussed. A physicist suggested that "bound water" be defined by a space-time correlation function. An astute observation was made that the reason everyone had a differing view of the amount of "bound" water and its definition was not a function of the system, but rather a function of the technique they used to make their measurements. In the study of water in bulk or in biological systems, there has always been an underlying problem. People who employ such techniques as neutron diffraction, Raman and infrared spectroscopy, and some dielectric relaxation, which look at the system over very short times ( $<10^{-12}$  sec), see a static picture of water. They argue for very little difference in hydrogen bond strengths and angles and water structure in cells versus bulk water. These same people favor the interpretation that only 1-3 layers of water near the surface show any difference at all. However, techniques which look at water on the kinetic time scale ( $>10^{-11}$  sec) (thermodynamics, NMR, ESR, dielectric relaxation, etc.) tend to suggest that the motional freedom of water is reduced in a significant way in living systems (5-20 layers of water).

Finney utilized his time on the panel to point out that thermodynamics data on biological systems are the only data whose interpretation is not model-dependent. Attempts to construct thermodynamics balance sheets, even for the folding of one protein, may fail, however, because of the hundreds of small interactions that occur between water and amino acid side chains. Based on our current understanding, "the sum of the parts is indeed less than the whole." Additional knowledge is needed on weak interaction chemistry before we can understand water's role in cells.

Dr. G. Careri (Inst. of Physics, Rome) pointed out that whatever these interactions are, the protein is very sensitive to them in performing its function. For lysozyme, critical hydration occurs at just the point where enough water molecules surrounded the protein to allow fluctuating movement (between 0.2 and 0.3 gm  $H_2O$ /gm protein). This hydration range for function was shown by several authors, including Dr. J. S. Clegg (Univ. of Miami, FL), for metabolic turn-on of brine shrimp.

Robson presented the results of Monte Carlo computer simulation for 350 water molecules around one amino acid. Even in such a simple



system, the effectiveness of the model depends on water-water interactions, which are poorly understood. When a nonpolar group is inserted into water, water structure in the vicinity of the surface is distorted. The question was asked as to why this distortion is not propagated out into the liquid. Two possible explanations were given: (1) the information for reorientation is simply damped out quickly by the fluid nature of water, (2) our current technologies are not capable of detecting weak orientational preferences.

Timasheff presented his conclusion that glycerol and other cosolvents are actually excluded from a volume of water near a protein surface. He pointed out that this effect did not correlate with any interaction parameter: the size of the protein, the size of the cosolvent molecule, or hydrophobicity. It correlated only with an increase in surface-free energy (or surface tension) of water around the protein surface area. He concluded that the hydration shell is an accidental consequence of water-cosolvent-protein interactions.

Dr. L. Rialdi (Inst. of Industrial Chemistry, Genoa) addressed the simple problem of how the addition of a small amount of a large polymer molecule, such as polyethylene glycol or polyoxyethylene, could cause the precipitation of a protein. Apparently, the action is by microscopic multipoint phase separation at the fixed charge sites of the protein as the polymer induces changes in water surface tension in the system.

These discussions pointed out that the concept of a fixed hydration shell of water around a protein probably is not a reality. The greatest effector of the water near the protein is the protein surface area and its charged nature. The addition of any other component into the system, such as salt ions, small chemicals, polymers, or other proteins, causes a complete redistribution of water interactions. However, these interactions may be of such a weak nature that current techniques find it difficult to measure them.

Finney presented the evening lecture on Monday, June 29, on the current state of the art in understanding the intermolecular nature of pure water. He pointed out that water is a fluid in constant motion, and that different techniques are employed to investigate the static geometric structure of water at any given instant in time, and the kinetics of mobility of the molecules. According to Finney, the absence of satisfactory theoretical models for the properties of pure water is due mostly to the weak flexible interactions between the molecules. He especially stressed that water cannot be modeled by a distorted ice lattice, since liquid water is denser than ice and the molecules must pack closer together. According to Finney, current models fail in attempting to explain the wide changes in the physical properties of water with temperature. He was extremely pessimistic about the use of Monte Carlo computer simulation until new calculations of the "pair potential," a critical term expressing how one water molecule is influenced by its neighbors, are accomplished.

#### MACROSCOPIC SURFACES AND HYDRATION INTERACTIONS

The plenary lecture on the second day was delivered by Dr. Barry

Ninham (Australian National Univ., Canberra). Ninham spoke about the way in which quantum mechanical calculations and various theoretical models explain experimental findings of the effects of surfaces on pure water and electrolyte solutions. He traced the development of various models for the interaction of two surfaces from the ideas of Laplace, Poisson, and Gibbs. Poisson maintained that there should be a density profile of interaction approaching an interface, contrary to other theories of abrupt changes very near the surface. According to Ninham, more modern theories of "Van der Waals and electrostatic forces based on continuum theories for biological systems show peculiarly delicate and subtle forms, presaging the onset of a rich hierarchy of behavior in the last 50 Å before the surface." One peculiarity of surface effects is that they may be oscillatory out from the substance. The major problem once again is the sensitivity of the technique used to measure the forces. The most probable conclusion is that significant surface effects can extend from 3 to 10 layers out into the water. The presence of salts can greatly alter these effects.

The posters presented on this topic, and their authors, were:

- (1) The Osmotic Properties of Hemoglobin under Physiological Conditions (B. Wittmann and G. Gros, Univ. of Essen, FRG); (2) The Role of Water in Phospholipid-Water Interactions (A. Bertoluzza et al., Italy); (3) Phospholipid Surface "Forces" and Hydration Studies by Differential Scanning Calorimetry DSC (L. Ter-Minassian-Saraga and G. Madelmont, CNRS, Paris); (4) Small Angle Neutron Scattering Study of Water Bound to a Protein (M.S. Lehmann and G. Zaccai, Inst. Lave-Langevin, Grenoble); (5) Theory of Hydration Forces in Ice (S. Marcelja et al., Austrian Nat. Univ., Canberra); (6) On the Role of Interfacial Water in Protonmotive Systems (D.B. Kell, Univ. of College of Wales); (7) Phospholipid Bilayer Hydration (R.P. Rand, Univ. of Nottingham, UK and V.A. Parsegian, Nat. Inst. of Health, Bethesda, MD); (8) Cooperativity and Propagation of a Structure Wave (J.G. Watterson, Univ. of Zurich); (9) The ordered Water Membrane Ion Channel (D.T. Edmonds, Clarendon Lab., Oxford, UK); (10) Free Energies at the Biosurface (D.J. McIver, S. Schurch, Univ. of Ontario, Canada); (11) Water/Ice Structure: Electrical Conductivity (D.C. Pressey, Clin. Res. Centre, Harrow, UK).

The poster of Wittman and Gros, which treated the classical problem of the non-ideal osmotic behavior of the red cell, was of special interest. Attempts have been made to explain the non-ideal swelling and shrinking of the cell by adding the osmotic coefficient of hemoglobin. However, this work showed that the postulated value of the coefficient necessary to describe the observations is significantly higher than the measured osmotic coefficient for hemoglobin. Remeasurement of the osmotic coefficient for hemoglobin by these authors brought the coefficient into closer agreement with experimental observations but still left some unexplained behavior of water near the hemoglobin surface and indicated that the amount of unfrozen water detected by NMR changes with the type of ionic solution used as a solute.

These reports focused on longer range effects on the physical properties of water out from surface. Most reports claimed a smooth and

continuously decreasing effect of a surface on water. Forces between lipid bilayers (similar to biological membranes) credited water as the major barrier to membrane fusion. Nonfreezing water accounted for 5-6 moles of  $H_2O$ /lipid head group. The chemical potential and the enthalpy of melting of water between bilayers is less than in bulk water. The possibility that water plays an important role in the mechanism of proton transport across the mitochondrial membrane was discussed.

In his poster depicting the ordered membrane water channel, Edmonds presented a theoretical model of an ion channel through the membrane which caused a great deal of discussion over the remaining days of the conference. He proposed that linear rods of protein line a channel through the membrane. In Edmond's interpretation, the inside of the channel is occupied by a network of water molecules in five (selective for  $Na^+$ ) or six membered rings (selective for  $K^+$ ). Ions move through the channel by jumping out of their hydration cage into a cage in the channel, and then from cage to cage through the channel. Such a system requires minimal movement of water, and the ion movement is driven by the electric field of the membrane potential. A portion of the discussion of Dr. Edmond's work is included here.

Franks: What causes the ion selectivity of your model channel?

Edmonds: Selectivity comes from the different sized water rings in the channels. A pentagon of five waters just fits  $Na^+$ , and a hexagon of six waters just fits  $K^+$ . These structures are optimum low energy states. In these structures water dipoles tip to hydrate the ion.

Lehmann: I'm always worried about dynamics, how much does this enter into your consideration?

Edmonds: The stability of this thing is due to the matching of the H bonds to the protein rods. Electron microscopy shows five and six rod configurations for acetyl choline stimulated ion channels. The size of the pores on EM is  $10A^\circ$ , very similar to the model, and the repetition distance is  $0.62-0.58 A^\circ$ , near  $0.6$  for the model. I don't know if this thing exists, but it is consistent with what hard information we have. With this model, one can explain the properties of the channels without gating particles. The function of the model is to suggest experiments. There are 5 to 6 experiments suggested by this model, now it's up to the experimentalists.

Maass: On your poster you show approximate agreement between the behavior required by your model and that of Hodgkin-Huxley. Could any differences be explained by your model not assuming a particular gating mechanism?

- Edmonds: I don't want to assume an extra gating mechanism. I think the gating mechanism is built into the structure of the channel. Channels don't open and shut, they exist in configuration A or B and change between the two in response to the electric field. Don't be too impressed by the agreement with Hodgkin-Huxley since both our models must satisfy the Nernst potential and are normalized to unity at zero membrane voltage.
- Hempling: My comment is that the acetylcholine sensitive site which is characterized by the structure which Dr. Edmonds uses for his model is different from the channel where specific gating of  $\text{Na}^+$  occurs and which leads to an action potential.
- Edmonds: I agree they are distinct types of channels. I used the acetylcholine channels because the molecular structure of the squid  $\text{Na}^+$  channel is not known. The major difference is that one is gated chemically and the other is electrically gated.
- Israelachvilli: Can you rationalize the unfavorable energetics of your model? You ask us to accept a hydrophilic surface with tight water and at the same time to forget water bound to the ion.
- Edmonds: The ion leaves its hydration shell behind and is locally hydrated by water in the channel, similar to ions traveling in water filled channels in clays. I prefer there be no translational motion of water in this channel, only rotational.
- Haglund: Under the electron microscope you can see two morphological types of channels. Can you estimate the ratio between the two types?
- Edmonds: I will give you some photographs and you can do it.
- Hempling: Do you propose separate channels for water movement?
- Edmonds: Trying to understand the role of water in this one channel has given me so much trouble, that I am going back to physics.

The formulation of models of such elementary functioning portions of the cell as ion channels through membranes requires the inclusion of water. The type of model developed by Edmonds requires a very specific sort of interaction of rigid protein rods coded by genetic messages with

water molecules of the environment to perform its function. The thermodynamics of cellular function, the building of macromolecular structures, and the dynamics of movements of solutes across cell membranes all still require consideration of the role of water molecules. Models of macromolecular structures or simplified electrical analogs are incorrect if they attempt to describe the system as a series of black boxes in which water plays no role. Scientists in this area of research are finding their services as collaborators in great demand by other groups seeking to understand their own experimental findings.

The panel discussion concentrated on the theoretical and experimental possibilities of long-range ( $>3$  layers) perturbations in water by surfaces. Some of the problems with the interpretation of NMR data from biological systems were discussed. The possibility that the nonrandom organization of the macromolecular matrix of cells may convey additional order on the system was considered. Specialized structures such as Edmonds' ion channels of  $\sim 200$  Å in length would "long range order." It was also pointed out by a theoretical physicist that "surface-induced dielectric polarization effects" can be quite small but very real beyond a few layers of water near a charged site. In live microsome spherical vesicles both nonfreezing and nonexchangeable water may be found. Evidence for surface effects of 100-500 Å was presented and some thermal anomalies in water properties were mentioned to generate discussion.

The Tuesday evening lecture was presented by Dr. J. Israelachvili (Australian Nat. Univ., Canberra) on Double-Layer, Van der Waals and Hydration Forces between Surfaces in Electrolyte Solutions. He has designed and built an extremely precise instrument for measuring repulsive and attractive forces between two smooth surfaces. Cylindrical shaped sheets of smooth mica are mounted on glass discs and brought together by an extremely accurate assembly of springs and screws inside a large volume of temperature-controlled electrolyte solution. The accuracy of placement is on the order of 0.1 Å and is monitored by a light beam passing through both sheets and producing a series of diffraction rings whose separation is a measure of distance and whose shape and color are indicative of the forces involved. In electrolyte solutions ions are apparently deposited on sites on the mica surface and retain a portion of their hydration shells which contribute to repulsive forces. At higher concentrations extra repulsive forces seem to be present between the plates. These forces are proportional to the hydrated radius of the atoms ( $\text{Li} > \text{Na} > \text{K} > \text{Cs}$ ). Upon bringing the plates very close together, the ions are apparently displaced by  $\text{H}^+$  ions. This can also be seen when pH is lowered. The subtraction of electrical double layer and Van der Waal forces leaves the forces due to hydration. This technique estimates such forces at 1 water molecule separation to be 1 KT and at 40-50 Å to be on the order of  $10^{-4}$  KT. The energy profile out from the surface decreases rapidly, yet Israelachvili cautioned that forces out 10 to 15 layers of water are still very real and might sum up to a significant effect in a high surface-to-volume-ratio system such as a living cell.

DYNAMICS OF WATER IN CELLULAR SYSTEMS

On the third day, the discussion moved from theoretical development and simple model systems to more complex real biological molecules and living cells. Dr. H. Hempling (Medical Univ. of South Carolina) reviewed the role of water as an agent of homeostasis in biological organisms. All cells contain large amounts of water, and under healthy conditions they are able to maintain a ratio of water to dry solids that is characteristic of their chemical maturity. During development, differentiation, and division, this ratio varies in a reproducible, genetically controlled manner. When subjected to an osmotic stress, cells may shrink or swell, but many types of cells will shortly return to their original size by a process called volume regulation, whose mechanism is not yet fully understood. In the whole organism, water in the circulatory system carries nutrients to cells and toxic wastes away. The high heat capacity of water in the body helps to maintain a constant body temperature in mammals and mediates temperature effects in other species. Water probably also plays a role in the genetic control of life processes by interactions with chromatin during transcription and translation.

Posters presented in this section were: (1) Water Transport in Cell Membranes of Plants (U. Zimmerman, IME Kernorschuneslage, FRG); (2) A Grotthus Mechanism in a Proton Conductor (J. Thomas, Chemistry Inst., Uppsala, Sweden); (3) Evidence for an Increase in Viscosity of Water Associated with Ion Pumping (P. Wiggins, Univ. of Auckland, New Zealand); (4) Cross-Linking Reagent Effects on Water and Ionic Flows in Cell Membranes (D.G. Margineau, Rumania); (5) Water Oxygen 17 Magnetic Relaxation in Aqueous Solutions of Macromolecules and Surfactant Micelles (B. Halle et. al., Chemical Center, Lund, Sweden); (6) Facilitated Proton Transfer in Protein Solutions by Rotational and Translational Protein Diffusion (G. Gros et al., Univ. of Essen, FRG); (7) Proton NMR Relaxation and Diffusion Study of Water Sorbed in Oriented DNA and Hyaluronic Acid (G. Lahajnar, Inst. Josef Stefan, Ljubljana, Yugoslavia); (8) Dynamics of Water in the Peripheral Nerve (V. Vasilescu, Rumania); (9) The Influence of Dynamic Properties of Water on Protein Fluctuations (R. Gregory and A. Rosenberg, Univ. of Minnesota); (10) Significance of Spin-Lattice Relaxation Times of Water Protons in Isolated Cell Nuclei to Pulsed Nuclear Magnetic Resonance Studies on Cancer (S.S. Ranade and S. Shah, India); (11) Physico-Chemical Essentials of Sodium and Potassium Recognition by Membrane Sodium Pump (V.A. Tverdislov, USSR).

A large fraction of these presentations concentrated on the role water may play in membrane structure and in membrane transport processes in living cells. A new pressure probe for single-cell measurements of turgor pressure in cells immobilized in a cross-linked polymer matrix was also discussed. The transport of  $H^+$  ions, which must exist as some form of water complex ( $H_3O^+$ , etc.) or move by proton tunneling across membranes, was treated as an important adjunct to new theories of proton motive force and  $H^+$  ion gradients driving other chemical reactions. The extensive use of NMR to monitor the mobility of water on surfaces of

macromolecules, in membrane structure, and in cytoplasm showed how powerful a nondestructive physical technique could be.

The afternoon panelists addressed the question of what one could learn from NMR about water in biological systems. Dr. W. Derbyshire (Univ. of Nottingham) explained that many investigators had sought a physical technique capable of studying water in a nondestructive manner in any kind of sample. NMR had met these criteria but suffered from the drawback of requiring extremely model-dependent interpretation. One interpretation would favor a model in which only a small portion of water in cells had lower mobilities than bulk water. The mean correlation time proposed by this model would be around  $10^{-9}$  sec (1c). Dr. P. Beall (Baylor College of Medicine, Houston) agreed that interpretation of NMR data was very model dependent. Any model proposed must address both  $T_1$  and  $T_2$  relaxation times of water protons, as well as the apparent real two-fold reduction in the self-diffusion coefficient of water in cells, and explain the differences in H, D, and O NMR findings. A continuum model with a declining profile of surface effects answers these points as well as a two-fraction model, and is more consistent with the findings of other physical techniques presented at this meeting. NMR is still of value for monitoring changes in the state of water in biological systems. Dr. K. Hallenga (Free Univ. of Brussels) utilized a  $^{17}\text{O}$  NMR technique to define an anisotropy in the motion of water molecules on surfaces that was in agreement with Dr. R. Bryant's (Univ. of Minnesota, Minneapolis) proton NMR findings. Dr. P. Wiggins (Univ. of Auckland) proposed that structurally restricted motion of water molecules in ion transporting channels undergoes transition to free states with macromolecular conformational changes elicited by ATP binding. This work is an attempt to integrate enzyme membrane ion pumps and ordered water structure in living cells.

#### PHYSIOLOGICAL WATER STRESS

The plenary lecture on the fourth day was given by Dr. F. Franks (Cambridge Univ.) on physiological water stress. This lecture extended the scope of the meeting into the area of the physiological significance of water structure in biological systems and the adaptive advantages biological organisms have utilized to deal with the physics of water in their cells. The adaptation of organisms to low temperature, freezing, and high salinity were discussed. Adaptations of amino acid sequences of proteins have occurred to provide stable enzymes at the temperature range of the organism. In most cells a certain fraction of water does not freeze even down to  $-70^\circ\text{C}$ . In other cells the freezing of water into ice crystals is prevented by special biochemicals that prevent the nucleation of ice. And other organisms are known which adapt to cold by utilizing the heat of fusion of ice formation to remain alive. Osmotic stresses are handled in different ways as well. Plant cell walls may mechanically resist osmotic pressure, and other cells may expend energy to remove excess ions actively from the cell or to synthesize osmoregulating solutes. The diversity of biological adaptation may offer experimental models to promote understanding of the properties of water in cells.

Posters presented in this section and their authors: (1) Reversible Dehydration of Membrane Vesicles (J. Crowe et al., Univ., of California, Davis); (2) Membrane Water and its Role in the Thermodynamic Model of Membrane Damage (J.J. McGrath, Michigan State Univ.); (3) Physical and Temporal Factors Involved in the Death of Embryos that Contain Ice (W.F. Rall et al., Agricultural Council, Cambridge, UK); (4) Fish Glycopeptide and Peptide Antifreezes: Their Interaction with Ice and Water (A.L. DeVries, Univ. of Illinois); (5) Evidence for a Protein Stabilizing Mechanism in Plant Cells during Water Stress Conditions (B. Schobert, Univ. of Munich, FRG); (6) The Biological Systems with Low Water Content (S. Ratkovic et al., Maize Res. Inst., Belgrade); (7) An Investigation into the Effects of growth under Water Stress in Sycamore Cells (H.W. Pritchard et al., N.E. London Polytech, UK); (8) Cellular Dehydration: Effects on the Plasma Membrane of Isolated Plant Protoplasts (P.L. Steponkus, Cornell Univ.); (9) NMR Relaxation Times of Water Protons in Cultured Cells during Freezing and under Osmotic Stress (P.T. Beall, Baylor College of Medicine, Houston); (10) Water Relations in the Epidermal Cells of the Halophyte (A.D. Tomos and R.G. Wyn Jones, Univ. Coll. of North Wales).

The poster on the use of glycopeptides by polar fishes to avoid freezing in  $-2^{\circ}\text{C}$  water, caused a great deal of discussion and intrigued the audience. The glycopeptides and peptides in the blood and tissues of the fishes depress the freezing point of water in the fish 200 times more than predicted by colligative relationships but have little effect on the melting point of ice. These molecules apparently adsorb to ice preventing nucleation of ice formation. It might be of interest to determine if these compounds could prevent ice formation in human tissues being stored for transplantation, or on equipment in cold climates. Another very interesting finding presented by Steponkus was that the classical view of cellular hydration may be wrong. From experiments with plant protoplasts (and those of others on epithelial-type cells) it seems that cells which slowly dehydrate under osmotic stress do not have greatly folded excess membrane. Membrane fragments are taken into the cell instead, thereby maintaining a spherical cell surface. Upon return to isotonic conditions, insufficient membrane surface is available to allow volume return and cells can lyse. The amount of cellular dehydration before freezing is a critical factor in whether ice will form in cells.

The panelists on environmental adaptation discussed those mechanisms that have evolved under stress conditions. Sometimes only one species occupies a particularly adverse environment, sometimes specialized enzymes with unusual temperature-dependent kinetics appear, even enzymes with special functions to aid in detoxification of the stressor evolve, and sometimes the organism just lives with the stress although at greatly reduced growth rates. Some organisms synthesize internal osmoregulators such as proline and glutamic acid to balance the high salt conditions of the environment. All of these adaptations seek to maintain conditions where water can fulfill its transport, structural, and lubricant roles.



ALTERNATIVE VIEWS ON THE ROLE OF WATER IN CELL FUNCTION

On the final day of the conference, Dr. J. Clegg (Univ. of Miami) presented the plenary lecture in which he posed the possibility that perturbations in water and ion properties in cells may be much more extensive and significant to biological function than hitherto conclusively demonstrated by physical techniques. The physical techniques discussed at this meeting demonstrated that surfaces can influence water properties, the major question being the range of those forces. Modern cellular anatomy utilizing instruments such as the fluorescent microscope and the million-volt electron microscope have demonstrated a complex interwoven network of filamentous proteins in living cells where spaces are small (100-1000 Å) and surface-to-volume ratios are very high. In rat liver cells, Clegg has estimated that membranes account for 95,000  $\mu^2$ . In these types of cells, fully 20% of cell water would be required to coat these surfaces with only 5 layers of water molecules. With all the other surfaces, molecular and ionic hydration shells, little water would be similar to bulk water. Clegg points out that most cellular biochemistry probably does not take place in the aqueous phase of the cell but rather on proteins in the matrix. One alternative view of the cell that incorporates a role for water and salts is the Association-Induction hypothesis of Dr. G. Ling. The cell is envisioned to be filled with a matrix of extended protein chains with charged surfaces. This matrix performs the function of ion and metabolite binding under the influence of cardinal absorbents such as ATP and  $Ca^{++}$  and also affects the solvent properties of water near its surfaces. The predictions of this theory are that ions and water in cells will not behave as if they were free as in a dilute solution. Ample evidence from Clegg, Horowitz, Garlid, Hempling, and others support the idea that the interior of the cell does not behave as if it were a dilute solution of all elements. Much more organization of macromolecular elements is seen and certain physical properties of ions and water suggest restricted mobilities. The major question for which an answer is being sought is whether these effects are small or whether they are large enough to be physiologically significant.

SUMMARY AND CONCLUSIONS

This meeting offered the first opportunity for scientists from around the world to live together for several days in an intensive, total-immersion environment to discuss their hypotheses and experimental results on the biophysics of water. It provided the setting for the greatest possible communication between the many interdisciplinary scientists interested in all aspects of water from the theoretical physics of ice to the proper conditions for the freezing of cells.

In the past 10 to 15 years, a dynamic group of international researchers interested in the biophysics of water has been created. This group is now able to apply numerous physical techniques including thermodynamics, nuclear magnetic resonance, dielectric relaxation, light scattering, neutron and X-ray diffraction, Raman and infrared spectroscopy, viscosity, isotope substitution, and measurements of freezing behavior

and force to the question of the physical properties of water in living systems. The physical measurements which have been made on model systems and living tissues leave no doubt that some of the physical properties of water are altered by surfaces in cells. The interior of the cell is not a dilute solution, and hypotheses based on such a model are physically and chemically incorrect.

The magnitude of surface effects on water in cells remains unclear. Static techniques reveal little or no differences in hydrogen bond angle, hydrogen bond strength, or the three-dimensional geometry of water in cells compared to bulk water. Measurable differences are detected by kinetic methods which reveal significant reductions in parameters which depend on the freedom of mobility of water molecules in cells. The propagation of surface effects into the surrounding water is best modeled by a decreasing energy profile out from the surface; a two-fraction model is no longer tenable. (The classification of three types of water, "bound," diffuse, and bulk, is not totally acceptable either).

The summation of many weak forces extending into the bulk of the cell from macromolecular surfaces may be sufficient to produce measurable differences in water properties. The physiological significance of alterations in the physical properties of water in cells is demonstrated by the nonfreezability of a portion of cell water, altered solvent properties for metabolites, structural contributions to  $H^+$  ion transport kinetics, evolutionary adaptation to water stress, and the failure of  $D_2O$  to substitute for  $H_2O$  in many life processes.

#### RECOMMENDED AREAS FOR FUTURE RESEARCH

The development of an improved theory of weak interaction chemistry would shed new light on the behavior of water in the complex system of a cell. In addition, the calculation of an improved "pair potential" is required before Monte Carlo computer simulation will yield useful information about a composite system of a molecule surrounded by water. New techniques are also needed which will bridge the gap between static geometric measurements and kinetic measurements on water (i.e., acoustic relaxation, laser light beat spectroscopy, and quasielastic neutron scattering will have an essential input). It is worth noting, too that more emphasis should be placed on the temperature and pressure dependence of water properties in biological model systems before physical theories can be developed.

Finally, an attempt must be made to define and measure the energies of interaction that result in the restriction of motional freedom. The energy profile needs to be defined for a surface approaching that of a real biological molecule, by numerous techniques.

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